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Award Number: W81XWH-11-1-0409

TITLE: Portable Low-Volume Therapy for Severe Blood Loss

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University of Minnesota Duluth
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REPORT DATE: June 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
June 2014

2. REPORT TYPE
Annual

3. DATES COVERED
09 May 2013 – 08 May 2014

4. TITLE AND SUBTITLE
Portable Low-Volume Therapy for Severe Blood Loss

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0409

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Biology
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
In our Year 2 Annual Report, we reported experiments described in Specific Aim 2. These included determining if outcomes from hemorrhagic shock in rats can be improved by combining BHB/M with the proven hypothermia-promoting adjunct 3-iodothyronamine (T1AM). This year, we explored the feasibility of administering a larger volume of a lower molarity BHB/M to hemorrhagically shocked rats as described in Specific Aim 3. Results from these experiments are described in this Annual Report for Year 3. In summary we made the following conclusions: 1.)*Melatonin provides therapeutic effects at very low concentrations*. This was shown by the low histopathological score observed when administering a solution containing melatonin at a concentration 10^{-6}-fold lower than that published by Klein *et al* [3]. 2.)*An isotonic form of BHB/M is not efficient for sustaining survival when compared to LR.* Lactated Ringers (LR) administration is more efficient at sustaining survival after 60% blood loss both at 24 hours and 10 days after surgery than any other treatment. LR provides essential mineral constituents of blood that isotonic BHB/M does not. 3.) *10-day survivors are on their way to full recovery.* The low histopathological scores for all the treatments administered suggest that if an animal has made it to 10 days it is not likely to develop multiple organ failure and die after the endpoint of our experiment.

15. SUBJECT TERMS
hemorrhagic shock, blood loss therapy, D-beta-hydroxybutyrate, melatonin

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	35	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

In our Year 2 Annual Report, we reported experiments described in Specific Aim 2. These included determining if outcomes from hemorrhagic shock in rats can be improved by combining BHB/M with the proven hypothermia-promoting adjunct 3-iodothyronamine (T1AM). This year, we explored the feasibility of administering a larger volume of a lower molarity BHB/M to hemorrhagically shocked rats as described in Specific Aim 3. Results from these experiments are described in this Annual Report for Year 3.

Body

Specific Aim 1: Dose ranging study of BHB/M components D-Beta-Hydroxybutyrate, Melatonin, and DMSO in hemorrhagically shocked rats

Histopathology

In the Year 2 Report we described the histopathological scoring system we developed for brain, lung, and small intestine (Table 1).

We have analyzed these tissues from the 10 day survivors of the melatonin dose-ranging experiments (Table 2). One-way ANOVAs with Tukey's *post hoc* test were performed to find treatment differences within each tissue. No statistical differences were observed for brain (Figure 1A) and lung (Figure 1B) tissues.

- *Small intestine*. The treatment with 4.3×10^{-5} mM melatonin exhibited greater histopathological damage than the treatment with 4.3 mM melatonin. However, the average score for the 4.3×10^{-5} mM melatonin treatment was just moderate (Figure 1C).

TNF-α

TNF-α was selected as a pro-inflammatory cytokine to assess inflammation. Since there were sample volume concerns, only one cytokine could be assessed. We chose TNF-α because for two reasons: 1) It has been reported that maximum changes in TNF-α levels occur within the first few hours of hemorrhagic shock[1], which coincides with the plasma samples we had available; and 2) TNF-α has been reported to differ from survivors and non survivors both in rat[1] and human[2] studies.

A pilot study has been conducted in order to discern whether melatonin affects inflammation in a dose-dependent manner. Four treatments and three time points were chosen. The treatments are: 1) 4 M BHB with 4.3 mM melatonin, 2) 4 M BHB with 4.3×10^{-5} mM melatonin, 3) 4 M NaCl with 4.3×10^{-5} mM melatonin, and 4) sham-operated animals. The time points ($T_{minutes}$) were: 1) after bolus infusion (T_{20}), 2) after 60% blood loss (T_{30}), and 3) one hour after 60% blood loss (T_{90}). TNF-α level were determined using an Invitrogen immunoassay (Model KRC3011. Life Technologies. Carlsbad, CA).Though the

experiment has been conducted, the data has not been analyzed yet and will be presented in our next quarterly report.

Specific Aim 3: Determine the feasibility of administering a larger volume of a lower molarity BHB/M to hemorrhagically shocked rats. Compare BHB/M with Lactated Ringer's solution in terms of promoting organ function and survival when administered as a non-sanguinous resuscitation fluid to hemorrhagically shocked rats pushed to failure

Experimental Design

Table 3 depicts our designed for isotonic formulations. Based on the results obtained from Specific Aim 1. We designed two isotonic BHB/M formulations, one with high (4.3 mM) melatonin and one with low (1.5×10^{-6} mM) melatonin. The latter being a direct dilution from the concentration optimized in Specific Aim 1. These formulations were compared to Lactated Ringer's solution (LR) alone and with the addition of 4.3 mM melatonin. Two surgical protocols were employed: one-hour shock (Figure 2A) and three-hour shock (Figure 2B).

Survival

Survival curves were compared at 24 hours and 10 days after 60% blood loss in SigmaPlot for Windows (version 11.0 Build 11.0.0.77) using a Gehan-Breslow-Wilcoxon test.

Three-hour Shock. The three hour shock protocol was conducted first as a pilot. *p*-values for all pairwise comparisons are summarized in Table 4 for 24 hours and Table 3 for 10 days. LR achieved better survival than the 140 mM BHB with 4.3 mM melatonin treatment at both 24 hours (Figure 3A) and 10 days (Figure 3B). However, it is worth mentioning that only 3 out of the 20 individuals survived to 10 days; the majority of deaths in all treatment groups occurred before 24 hours. For this reason, this study was left as a pilot with a sample size of 5 per treatment.

One-hour Shock. No statistical differences were observed at 24 hours (Figure 4A; Table 5). However, it is worth mentioning that only 3 out of 10 animals in the 140 mM

BHB with $1.5x10^{-6}$ mM melatonin treatment made it to 24 hours. At 10 days, both LR and LR plus 4.3 mM melatonin showed a greater survival benefit when compared to the 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment, but not the 140 mM BHB with 4.3 mM melatonin treatment (Figure 4B; Table 5).

Blood Gas Data

Blood was collected at specific time points throughout the hemorrhagic shock protocol. These samples were analyzed in a blood gas analyzer (BGA) ABL815 Flex (Radiometer America). One-way ANOVAs with Tukey's *post hoc* test were performed to find treatment differences within different time points for total hemoglobin (tHb), pH, saturation of oxygen (sO_2), potassium ion (K^+), sodium ion (Na^+), calcium ion (Ca^{++}), chloride ion (Cl^-), glucose (Glu), and lactate (Lac).

Three-hour Shock. Blood samples were collected at six time points: 1) before hypotension (T_0), 2) after 40% blood loss (T_{10}), 3) ten minutes after 40% blood loss (T_{20}), 4) after 60% blood loss (T_{30}), 5) three hours after 60% blood loss (T_{210}) and 6) after blood return (T_{225}). No statistical differences were observed for tHb, pH, sO_2, and Glu. Figure 5 shows all the data obtained from the blood gas analyzer. All observations for each parameter have been summarized in Table 6.

- **K^+.** At T_{210} and T_{225}, both LR and LR plus 4.3 mM melatonin had higher K^+ levels than either of our isotonic formulations of BHB/M. Also at T_{225}, plasma concentrations of K^+ were higher in the LR plus 4.3 mM melatonin treatment than in LR alone.
- **Na^+.** LR had higher Na^+ levels than the 140 mM BHB with 4.3 mM melatonin treatment at T_{210} and T_{225}.
- **Ca^{++}.** At T_{210}, both LR and LR plus 4.3 mM melatonin had higher Ca^{++} levels than either of our isotonic formulations of BHB/M. At T_{225}, LR had higher Ca^{++} than either of our isotonic BHB/M formulations; LR plus 4.3 mM melatonin had higher Ca^{++} levels than the 140 mM BHB with 4.3 mM melatonin treatment.
- **Cl^-.** At T_{210} and T_{225}, both LR and LR plus 4.3 mM melatonin had higher Cl^- levels than either of our isotonic formulations of BHB/M.

- **Lac.** The 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment had higher Lac values than LR at T_0 and T_{30}. At T_{20}, it also had higher Lac levels than LR and LR plus 4.3 mM melatonin. These observations are at time points where no infusion has occurred yet and depict strong individual variation more than treatment differences.

One-hour Shock. Blood samples were collected at six time points: 1) before hypotension (T_0), 2) after 40% blood loss (T_{10}), 3) ten minutes after 40% blood loss (T_{20}), 4) after 60% blood loss (T_{30}), 5) one hour after 60% blood loss (T_{90}) and 6) after blood return (T_{105}). No statistical differences were observed for pH, Na^+, and Glu. Figure 6 and Table 7 summarize these data.

- **tHb.** At T_{30}, tHb was lower in the 140 mM BHB with 4.3 mM melatonin treatment compared to the LR plus 4.3 mM melatonin and 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatments. At T_{90}, the LR plus 4.3 mM melatonin treatment was lower than the 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment.
- **sO_2.** LR plus 4.3 mM melatonin was more successful at maintaining blood oxygen saturation than LR alone at T_{90}.
- **K^+.** At $T_{105,}$ the 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment had lower K^+ levels than both the LR and LR plus 4.3 mM melatonin treatments; the 140 mM BHB with 4.3 mM melatonin treatment had lower K^+ levels when compared to the LR plus 4.3 mM melatonin treatment.
- **Ca^{++}.** All other treatments had higher levels of Ca^{++} than the 140 mM BHB with 4.3 mM melatonin treatment at T_{90}. Treatment differences became more evident at T_{105} since the animals treated with LR had higher plasma Ca^{++} than those administered with either of our isotonic formulations of BHB/M. Also at T_{105}, LR plus 4.3 mM melatonin had higher Ca^{++} levels than the 140 mM BHB with 4.3 mM melatonin treatment.
- **Cl^-.** The 140 mM BHB with 4.3 mM melatonin treatment had lower Cl^- levels when compared to the LR plus 4.3 mM melatonin treatment at T_{90} while the

140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment had lower Cl^- levels than both the LR and LR plus 4.3 mM melatonin treatments.

- **Lac.** At T_{105}, LR plus and without melatonin had higher Lac levels than the 140 mM BHB with 4.3 mM melatonin treatment.

Regression Analyses

Cox regression analyses were performed in SAS 9.4 using a phreg procedure to elucidate whether survival was affected by any of the parameters measured by the BGA regardless of the treatment administered.

Three-hour Shock. We found that pH, tHb, sO_2, K^+, Na^+, Ca^{++}, Cl^-, Glu and Lac do not affect survival at any time point (Table 8).

One-hour Shock. A summary of the regression results can be found in Table 9. pH, tHb, sO_2, K^+, Na^+, Ca^{++}, and Lac do not seem to influence survival at any time point.

- **Cl^-.** At T_{90}, Cl^- levels may influence survival.
- **Glu.** Glu levels at T_{10} appear to have an effect in survival.

PowerLab Data

Physiological parameters such as mean arterial blood pressure (MAP), heart rate (HR), and rectal temperature were monitored during the whole procedure using PowerLab 30/4 (ADInstruments). One-way ANOVA with Tukeys *post hoc* tests were conducted for mean arterial blood pressure (MAP), heart rate (HR), and rectal temperature.

Three-hour Shock. Eight time points were observed: 1) before hypotension (T_0), 2) after 40% blood loss (T_{10}), 3) ten minutes after 40% blood loss (T_{20}), 4) after 60% blood loss (T_{30}), 5) one hours after 60% blood loss (T_{90}), 6) two hours after 60% blood loss (T_{150}), 7) three hours after 60% blood loss (T_{210}) and 8) after blood return (T_{225}). These data can be found in Figure 7 and Table 10. No statistical differences were observed for HR and rectal temperature.

- **MAP.** At T_{90}, LR was able to sustain a higher MAP than the 140 mM BHB with $1.5x10^{-6}$ mM melatonin treatment. At T_{225}, LR proved more effective in maintaining a higher MAP than the LR plus 4.3 mM melatonin treatment.

One-hour Shock. Six time points were observed: 1) before hypotension (T_0), 2) after 40% blood loss (T_{10}), 3) ten minutes after 40% blood loss (T_{20}), 4) after 60% blood loss (T_{30}), 5) one hour after 60% blood loss (T_{90}) and 6) after blood return (T_{105}). Figure 8 and Table 11 summarize these data.

- **MAP.** The LR plus 4.3 mM melatonin treatment had lower MAP at T_{90} and T_{105} compared to all other treatment groups.

Regression Analyses

As with BGA data, Cox regression tests between PowerLab data and survival were performed with the objective of clarifying whether MAP, heart rate, or rectal temperature influenced survival independently of the treatment administered.

Three-hour Shock. MAP, heart rate, and rectal temperature at any time point did not seem to influence survival (Table 12).

One-hour Shock. Table 13 summarized the results of the regression analyses for PowerLab data. Rectal temperature did not seem to influence survival.

- **MAP.** At T_{20}, MAP appears to exert influence on survival.
- **HR.** HR also seems to impact survival at T_{20}.

Histopathology

Brain, lung, and small intestine from rats that survived to 10 day in the one-hour Shock experiments were analyzed using a one-way ANOVAs with Tukey's *post hoc* test in order to discern treatment differences within each tissue. No statistical differences were observed for brain (Figure 9A), lung (Figure 9B), or small intestine (Figure 9C).

Key Research Accomplishments

- Completion of histopahological analysis for Specific Aim 1.
- Pilot measurement of TNF-α for Specific Aim 1.
- Completion of isotonic formulation experiments
- Completion of histopathological analysis for Specific Aim 3

Reportable Outcomes

On April 12th, 2014, the graduate student conducting the experiments, Cecilia Edna Pérez de Lara Rodríguez, presented a poster at the first Aufderheide Memorial Lecture and Student Research Symposium hosted by the Biomedical Science Department of the School of Medicine of the University of Minnesota Duluth.

Postdoctoral researcher Christine Schwartz studied the mechanism of the neuroprotective aspects of melatonin using the specific melatonin receptor antagonist luzindole in hibernating thirteen-lined ground squirrels. Hibernating mammals show natural neuroprotection and can provide additional clues on how we can improve the current BHB/M blood loss therapy. This data was presented by Dr. Schwartz at the FASEB conference on "Melatonin Biology: Actions and Therapeutics" held July 7th-12th, 2013 in Niagara Falls, NY. (Schwartz, C., Perez de Lara Rodriguez, C.E., and Andrews, M.T. (2013). Melatonin as a protective component of the hibernation-based blood loss therapy, BHB/M. FASEB Conference: Melatonin biology: Actions and therapeutics, July 7th-12th, 2013, Niagara Falls, NY.)

In an effort to further optimize the portable therapy for blood loss, postdoctoral researcher Dr. Katie Vermillion will be studying heart function under physiological extremes in hibernating ground squirrels. She has begun to perform proteogenomic experiments using hibernating and active ground squirrels that are naturally subjected to physiological extremes resembling hemorrhagic shock. Her preliminary findings will be presented at the 62nd ASMS Conference on Mass Spectrometry and Allied Topics in Baltimore, MD on June 15th-19th, 2014.

Conclusion

Specific Aim 1

- *<u>Melatonin provides therapeutic effects at very low concentrations</u>*. This is evident by the low histopathological score observed when administering a solution containing melatonin at a concentration 10^{-6}-fold lower than that published by Klein *et al* [3].

Specific Aim 3

- *<u>An isotonic form of BHB/M is not efficient for sustaining survival when compared to LR.</u>* LR administration, is more efficient at sustaining survival after 60% blood loss both at 24 hours and 10 days after surgery than any other treatment. This may be because parenteral fluids should have a similar composition to normal plasma[4]. LR provides essential mineral constituents of blood which isotonic BHB/M does not.
- *<u>10-day survivors are on their way to full recovery.</u>* The low histopathological scores for all the treatments administered suggest that if an animal has made it to 10 days it is not likely to develop multiple organ failure and die after the endpoint of our experiment. This is only true for our rat model.

So What?

The surgical experiments for Specific Aim 3 have been concluded. Survival, Blood Gas Analyzer, PowerLab data, and histopathological scores have been analyzed and are presented in this document.

No strong conclusions can be drawn from the Three-hour shock experiments because the overall survival to 10 days with our large volume resuscitation experiments is 15%. It is evident that the insult of 60% blood loss combined with 3 hours without a blood transfusion is too great for any resuscitation fluid to counteract.

With the one-hour shock experiments it became evident that the current standard of care, LR administration, is more efficient at sustaining survival after 60% blood loss both at 24 hours and 10 days after surgery than any other treatment. Although there were no electrolyte effects on survival, LR, with or without melatonin, provide better electrolyte support than 140 mM BHB with 1.5×10^{-6} mM Mel or 140 mM BHB with 4.3 mM Mel as evident by the fact that the treatments containing LR maintained higher values for K^+, Na^+, Ca^{++}, and Cl^-, regardless of statistical significance. We speculate that the administration of a mixture of electrolytes that is similar to that of the plasma is important for sustained survival when the resuscitation strategies involve isotonic fluids.

The addition of high melatonin (4.3 mM) to LR was comparable in survival to LR on its own at 24 hours but failed at sustaining that survival to 10 days. It is interesting that the addition of melatonin to LR resulted in lower survival, even if not statistically significant. It is possible that the interaction of melatonin with the components of LR differs from its interaction with the components of BHB/M.

To further optimize BHB/M we plan to continue to study the effect of melatonin in preserving mitochondrial function in the naturally hibernating thirteen-lined ground squirrel, *Ictidomys tridecemlineatus*. These studies will concentrate on the mitochondria-rich brown adipose tissue and the normally ischemic-sensitive heart and brain. We began animal studies following December 31st, 2013 approval of the ACURO application.

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3. Klein, A.H., et al., *Small-Volume d-ß-Hydroxybutyrate Solution Infusion Increases Survivability of Lethal Hemorrhagic Shock in Rats.* Shock, 2010. **34**(6): p. 565-572 10.1097/SHK.0b013e3181e15063.
4. Hartmann, A.F., *Theory and practice of parenteral fluid administration.* Journal of the American Medical Association, 1934. **103**(18): p. 1349-1354.

Appendices

Figures

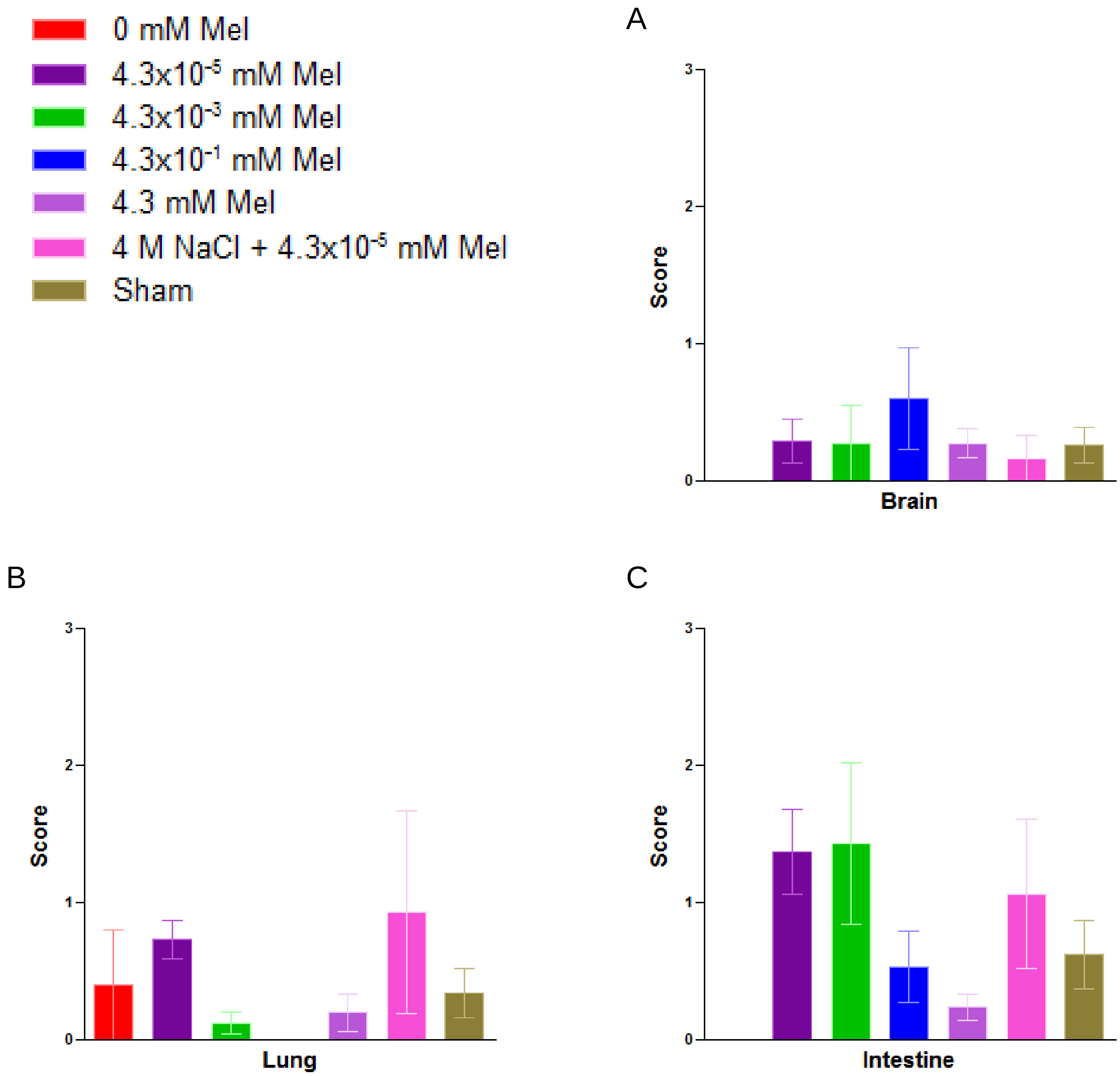


Figure 1. Histopathological scores. Melatonin Dose-Ranging Study. A. Brain. B. Lung. C. Intestine. All treatments include 4 M BHB and 2% DMSO except NaCl .000043 mM Mel (4M NaCl/.000043 mM Mel/2% DMSO). Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin. DMSO=dimethyl sulfoxide.

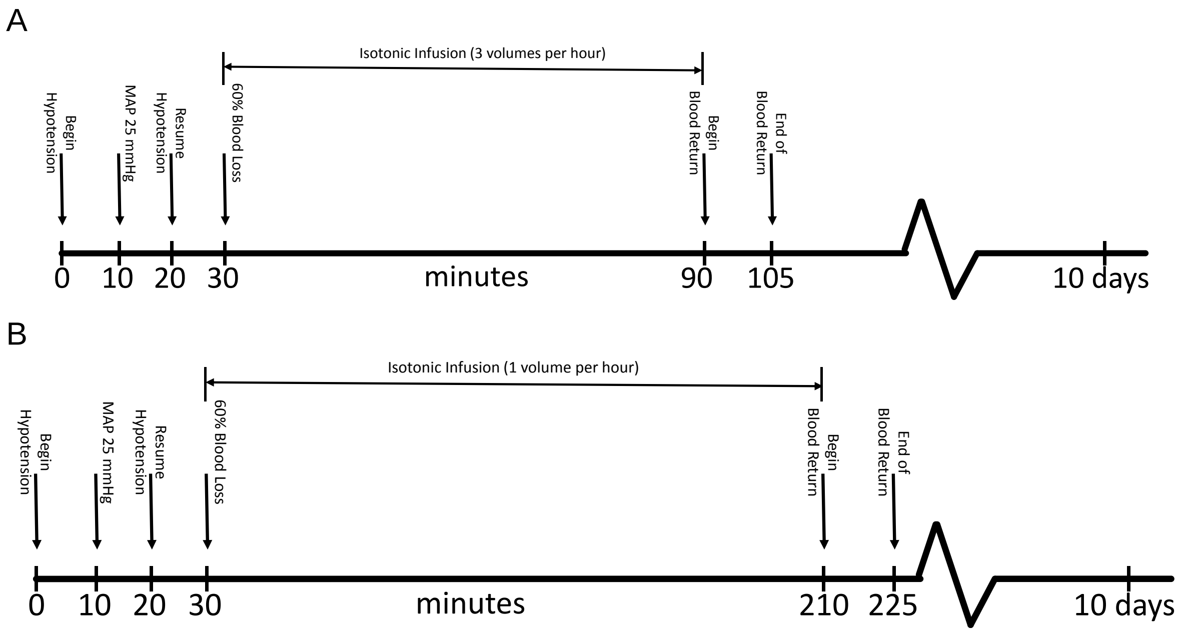


Figure 2. Hemorrhagic Shock Protocol. A. One-Hour Shock. B. Three-Hour Shock.

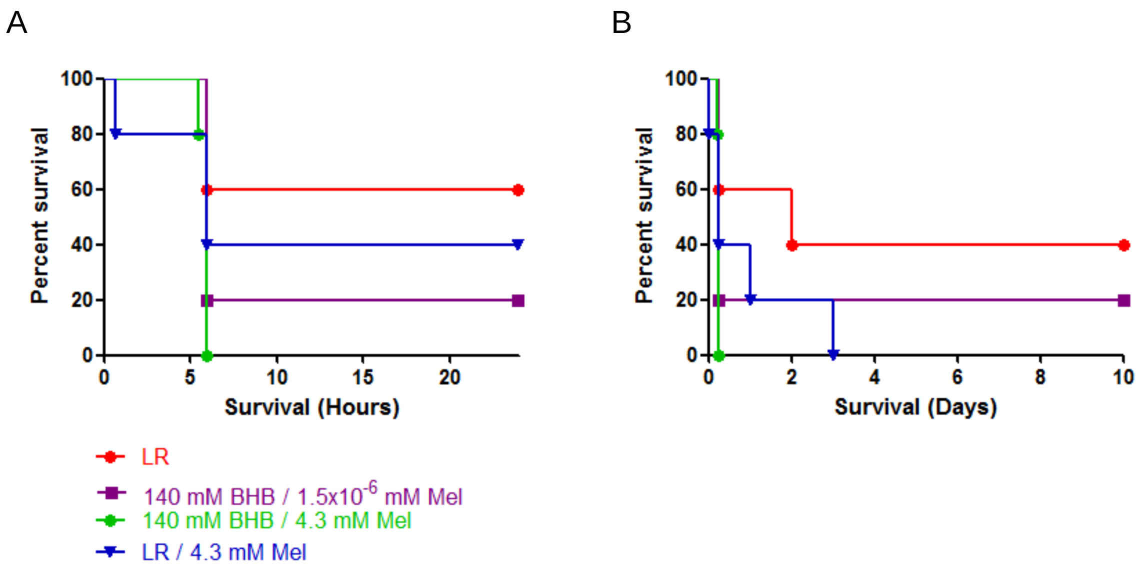


Figure 3. Kaplan-Meier Survival Curve. Three-Hour Shock. A. 24 hours. B. 10 days. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.

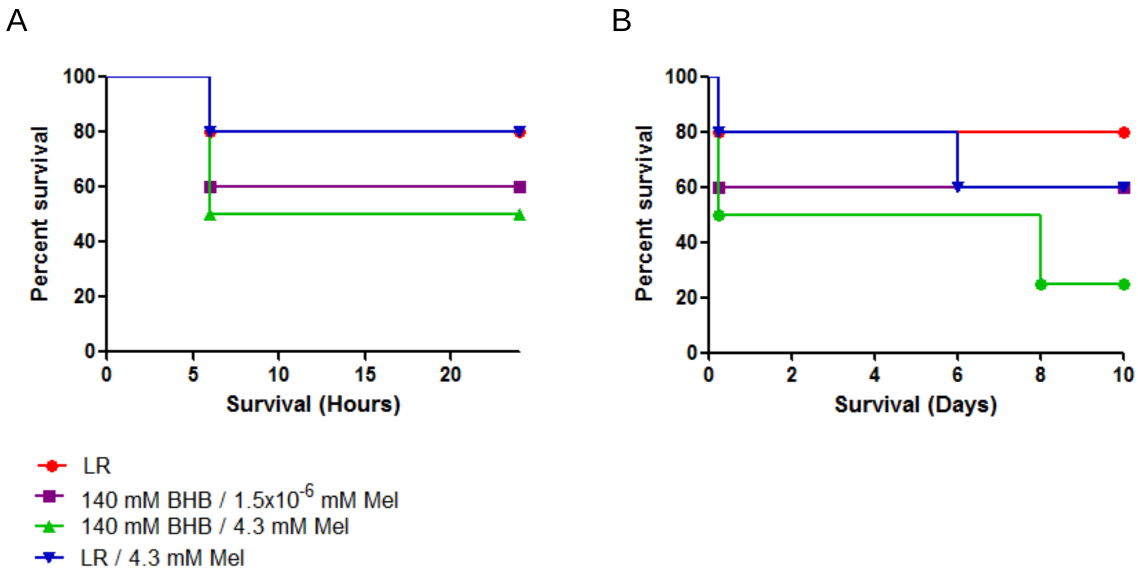


Figure 4. Kaplan-Meier Survival Curve. One-Hour Shock. A. 24 hours. B. 10 days. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.

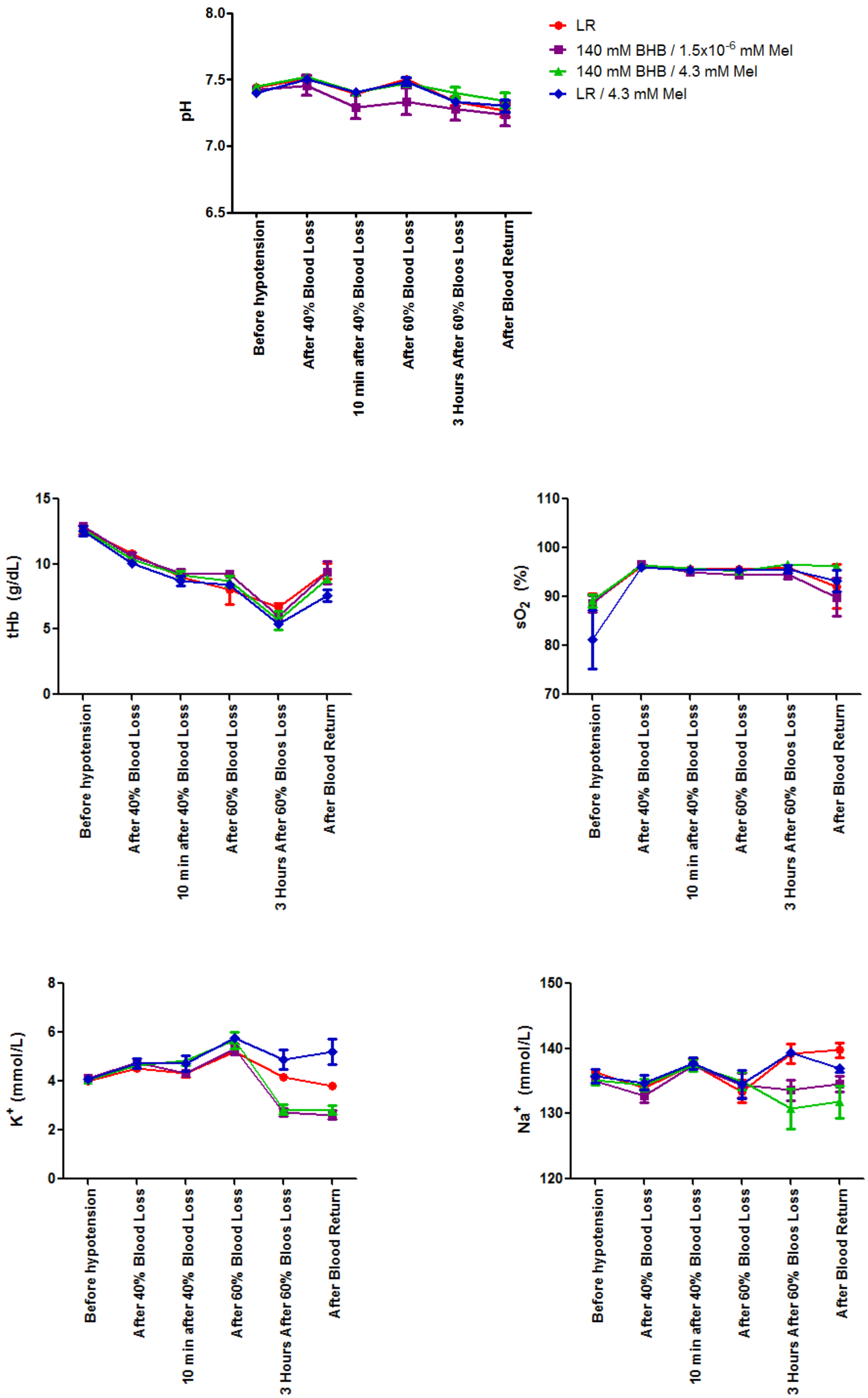


Figure 5. Blood Gas Data. Three-Hour Shock. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.

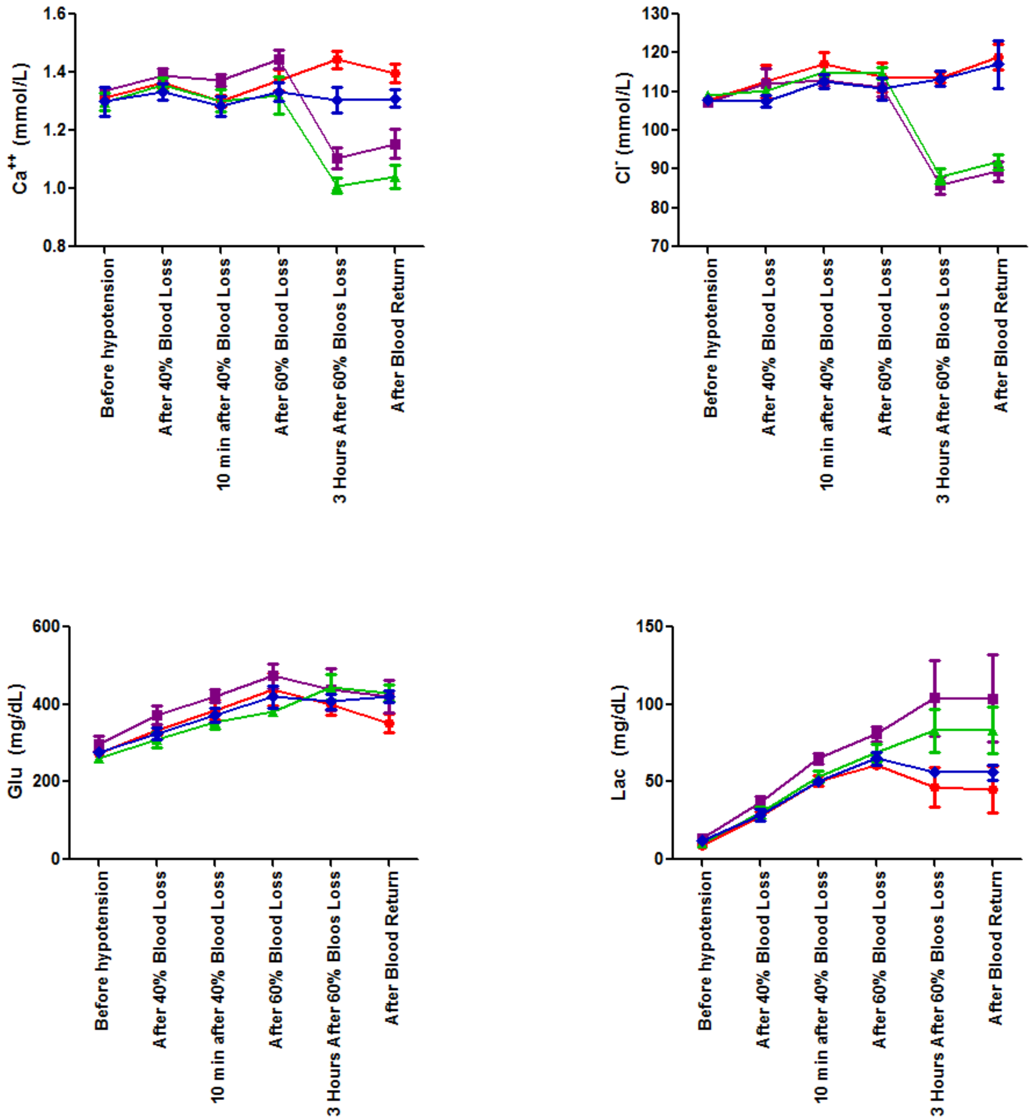
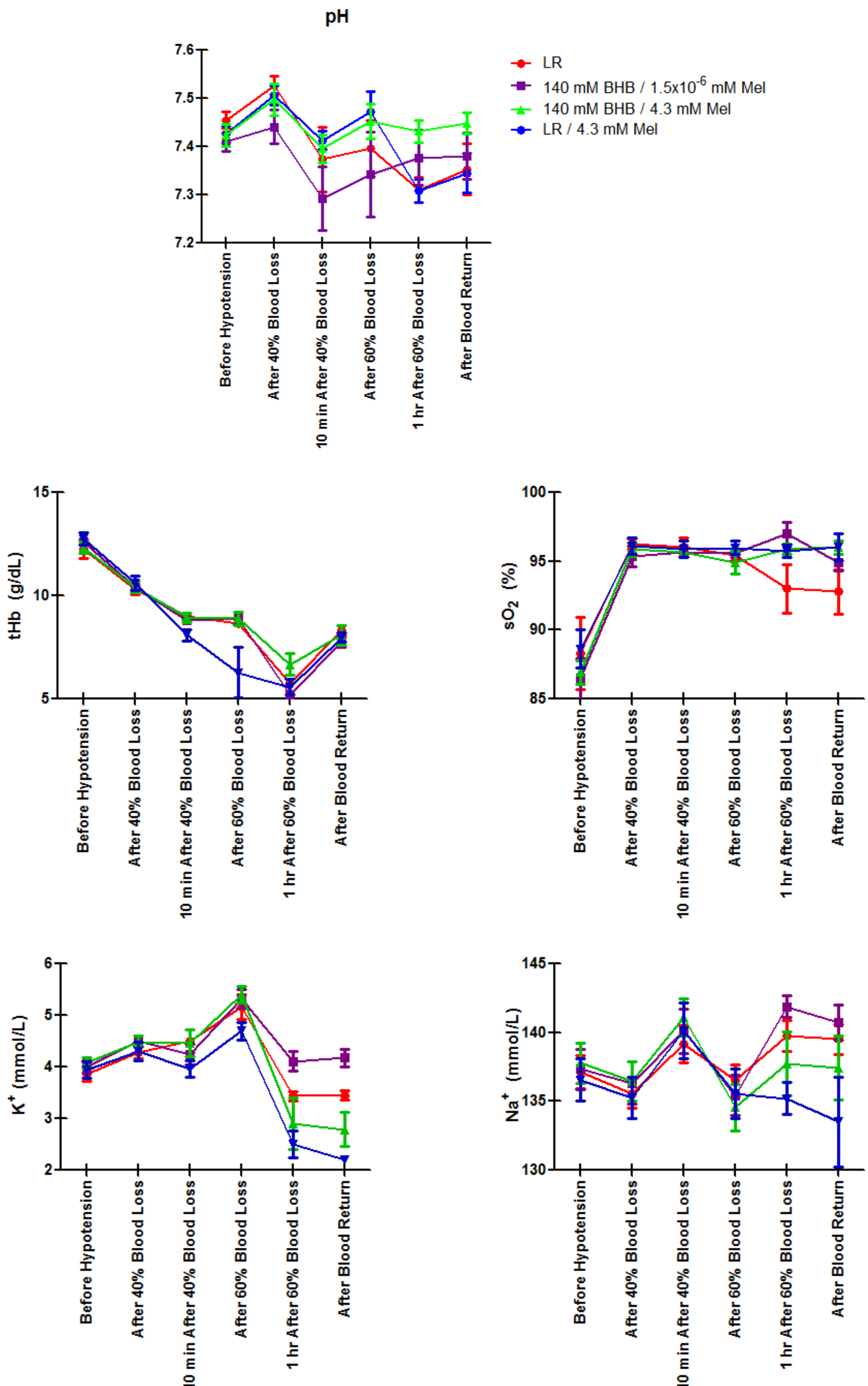


Figure 5 (Continued). Blood Gas Data. Three-Hour Shock. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.



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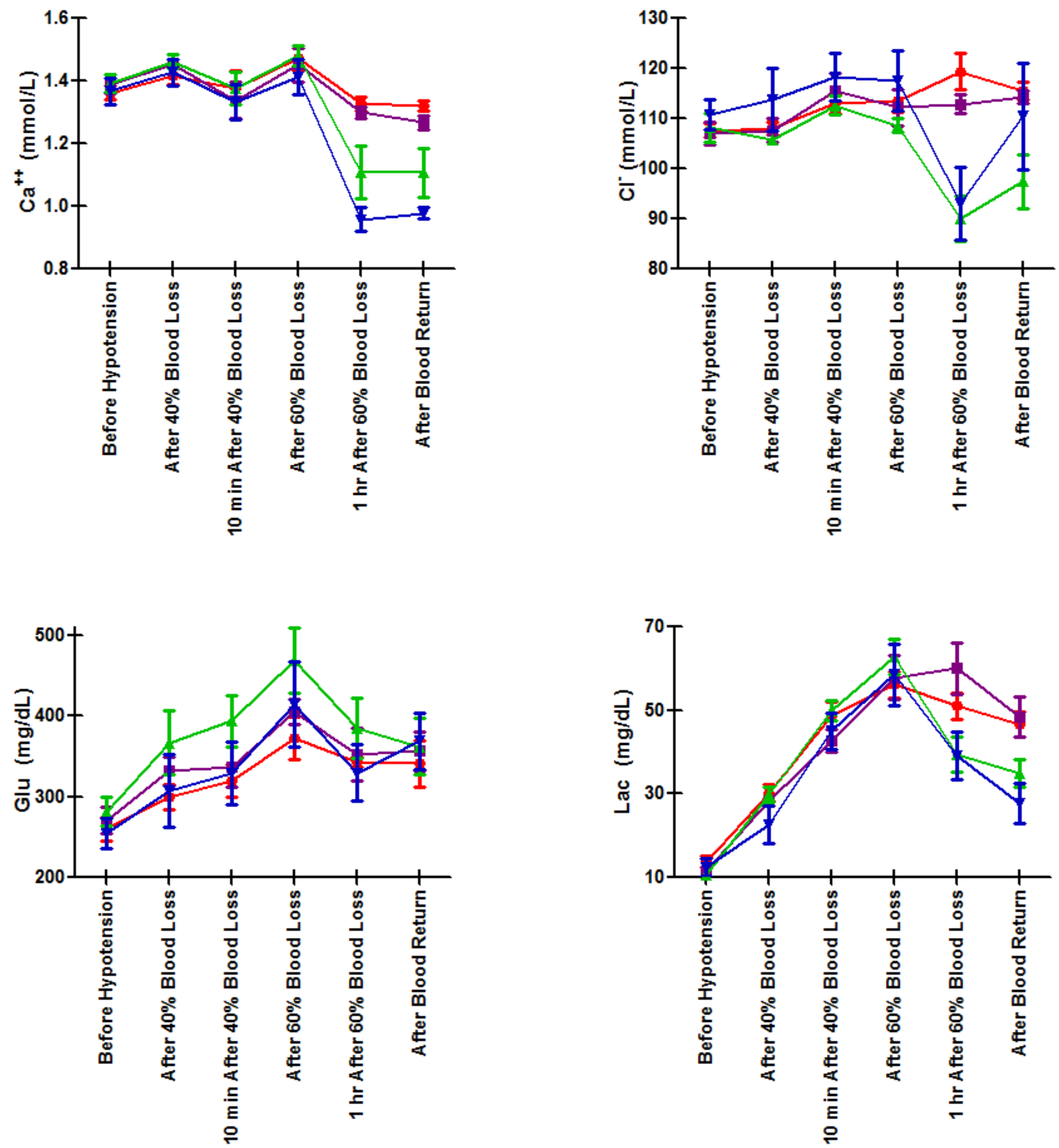

Figure 6 (Continued). Blood Gas Data. One-Hour Shock. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.

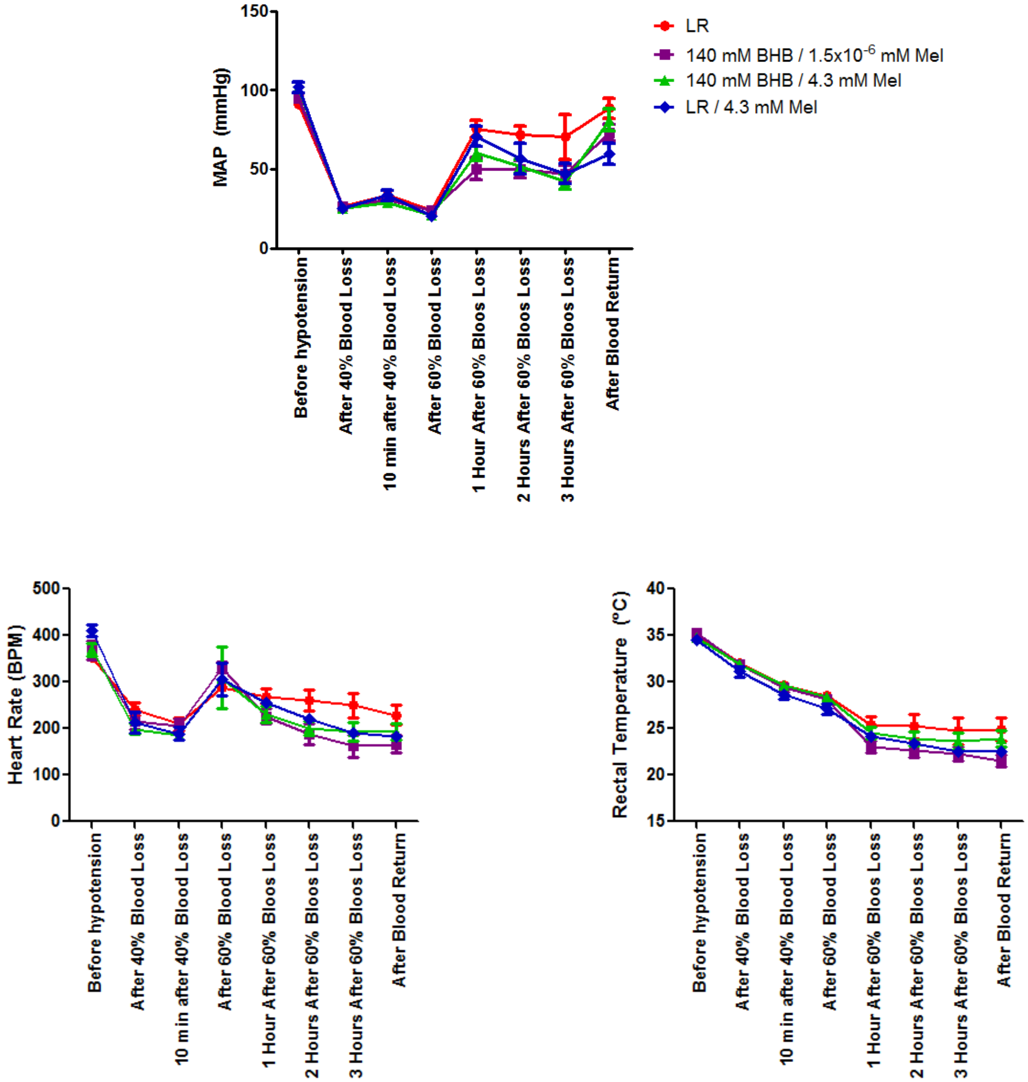

Figure 7. PowerLab Data of Mean Arterial Blood Pressure, Heart Rate, and Rectal Temperature. Three-Hour Shock. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin. MAP=mean arterial pressure. BPM=beats per minute.

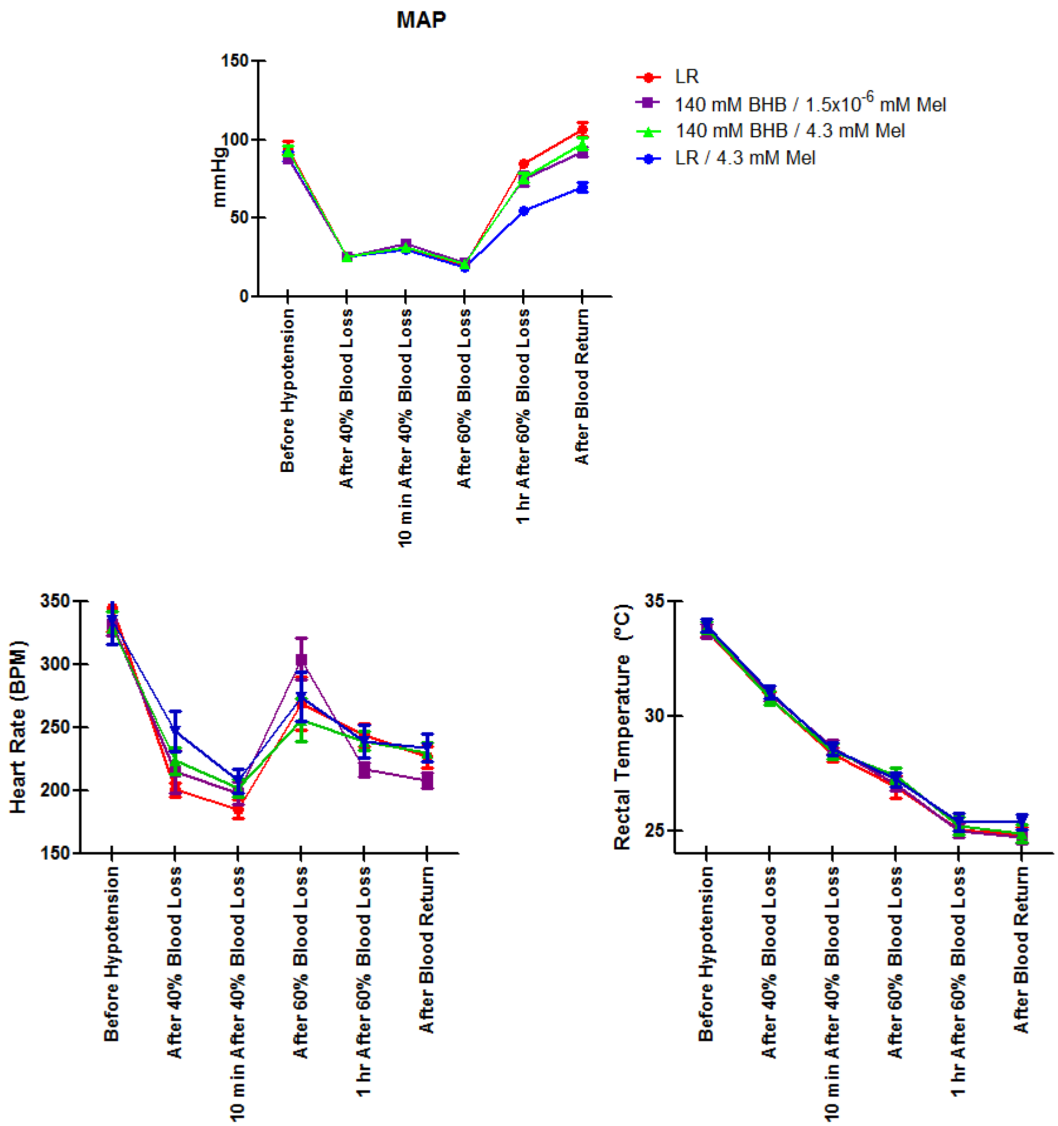

Figure 8. PowerLab Data of Mean Arterial Blood Pressure, Heart Rate, and Rectal Temperature. One-Hour Shock. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin. MAP=mean arterial pressure. BPM=beats per minute.

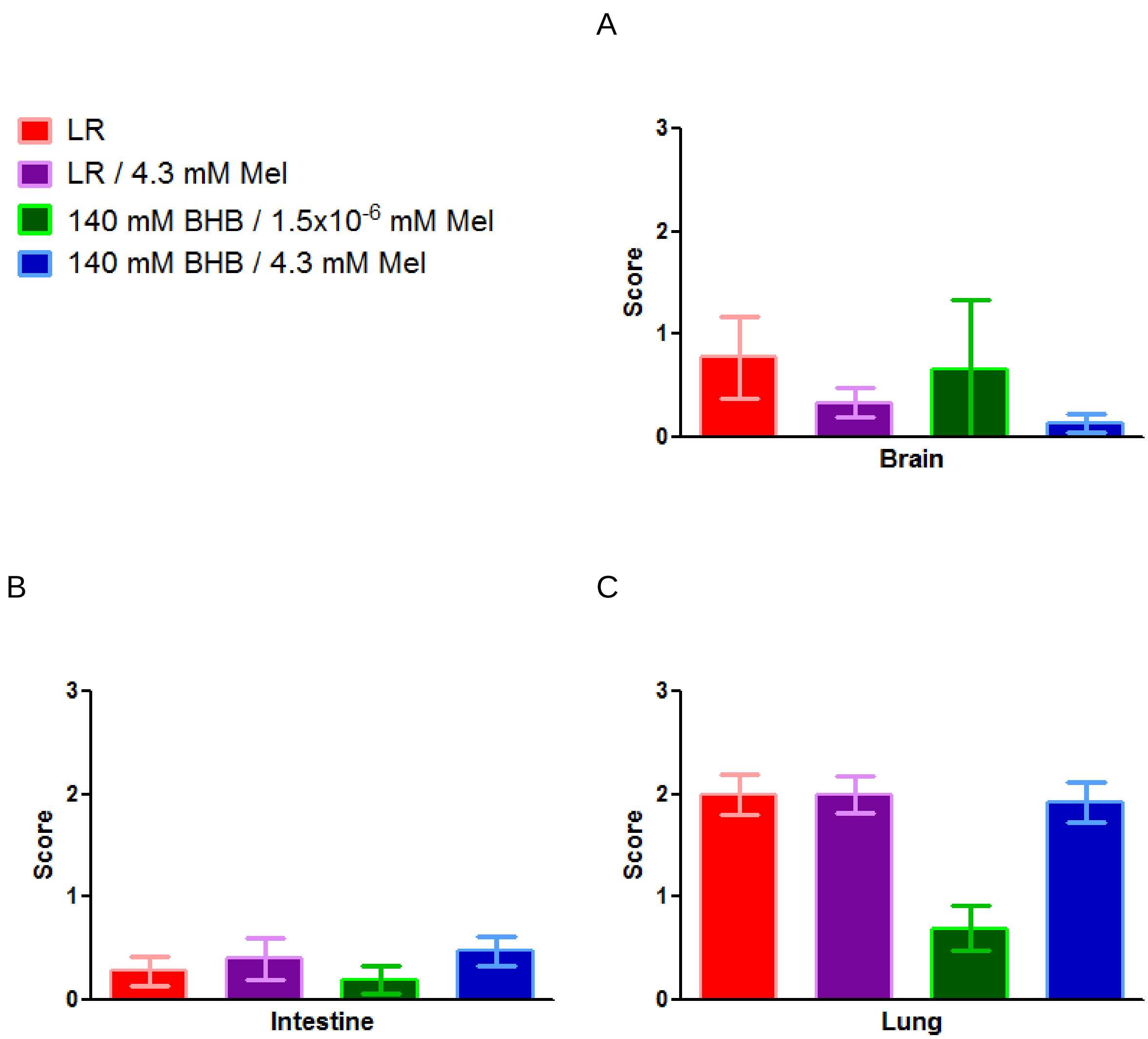

Figure 9. Histopathological scores. One-Hour Shock. A. Brain. B. Lung. C. Intestine. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin.

Tables

Table 1. Histopathological scoring system.

		Lung	Intestine	Brain
0	No Evidence			
1	Mild	Alveolitis (2-3X), Perivascular edema	Development of subepithelial Gruenhagen's space, vacuolization at the villus tip	Focal pyknosis
2	Moderate	Alveolitis (3-4X), Interstitial edema	Lifting of epithelial layer from the lamina propria, Increased vacuolization from the tip to midportion of villi,	Multifocal pyknosis
3	Severe	Alveolitis (>5X), Alveolar edema, Inflammatory infiltrate, Hemorrhage	Epithelial lifting and vacuolization from the tip to lower portion of villi, Mucosal ulceration and disintegration of the lamina propria, Inflammatory infiltrate, Hemorrhage	Extensive pyknosis

Table 2. Melatonin dose-ranging design. Abbreviations: BHB=D-β-hydroxybutyrate. Mel=melatonin. DMSO=dimethyl sulfoxide.

	Mel (mM)	DMSO
4 M BHB	4.3	2%
4 M BHB	$4.3x10^{-1}$	2%
4 M BHB	$4.3x10^{-3}$	2%
4 M BHB	$4.3x10^{-5}$	2%
4 M BHB	None	2%
4 M NaCl	4.3x10-5	2%

Table 3. Isotonic formulation design.

Component	LR (n=10)	LR / 4.3 mM Mel (n=10)	140 mM BHB / 1.5×10^{-6} mM Mel (n=10)	140 mM BHB / 4.3 mM Mel (n=10)
D-β-hydroxybutyrate			140 mM	140 mM
D-L-lactate	28 mM	28 mM		
Sodium	130 mM	130 mM	140 mM	140 mM
Potassium	4 mM	4 mM		
Calcium	3 mM	3 mM		
Chloride	109 mM	109 mM		
Melatonin		4.3 mM	1.5×10^{-6} mM	4.3 mM
DMSO		0.2%	0.02%	0.02%

Table 4. Survival. p-values of pairwise comparisons of 3-hour shock experiments. Statistically significant values are marked in red.

Treatment Comparisons	24 Hrs	10 Days
LR vs LR plus 4.3 mM melatonin	0.419	0.236
LR vs 140 mM BHB with 1.5×10^{-6} mM melatonin	0.221	0.281
LR vs 140 mM BHB with 4.3 mM melatonin	0.042	0.042
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.905	0.907
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.411	0.411
140 mM BHB with 1.5×10^{-6} mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.180	0.236

Table 5. Survival. p-values of pairwise comparisons of 1-hour shock experiments. Statistically significant values are marked in red.

Treatment Comparisons	24 Hrs	10 Days
LR vs LR plus 4.3 mM melatonin	0.957	0.691
LR vs 140 mM BHB with 1.5×10^{-6} mM melatonin	0.064	0.022
LR vs 140 mM BHB with 4.3 mM melatonin	0.726	0.466
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.064	0.035
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.726	0.678
140 mM BHB with 1.5×10^{-6} mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.081	0.052

Table 6. BGA Data. p-values of pairwise comparisons of 3-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	Before Hypotension								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.247	0.990	0.296	0.888	0.924	0.980	1.000	1.000	0.102
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.845	0.926	1.000	0.713	0.510	0.899	0.945	0.563	0.010
LR vs 140 mM BHB with 4.3 mM melatonin	0.991	1.000	1.000	0.990	0.629	0.957	0.693	0.885	0.482
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.645	0.815	0.329	0.991	0.890	0.764	0.963	0.626	0.732
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.160	0.985	0.270	0.972	0.952	1.000	0.704	0.889	0.688
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.689	0.940	0.999	0.870	0.997	0.641	0.380	0.214	0.148
	After 40% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	1.000	0.236	1.000	0.759	0.941	0.828	0.692	0.979	0.998
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.807	0.911	0.943	0.621	0.774	0.837	0.999	0.464	0.285
LR vs 140 mM BHB with 4.3 mM melatonin	0.993	0.603	0.996	0.927	0.988	0.997	0.945	0.738	0.977
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.851	0.538	0.921	0.998	0.479	0.421	0.765	0.312	0.422
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.991	0.852	0.989	0.976	0.993	0.900	0.935	0.937	0.997
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.655	0.931	0.987	0.927	0.590	0.736	0.975	0.097	0.486
	10 min After 40% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.998	0.881	0.999	0.511	0.999	0.975	0.442	0.972	1.000
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.465	0.884	0.836	1.000	0.998	0.289	0.441	0.563	0.022
LR vs 140 mM BHB with 4.3 mM melatonin	0.993	0.975	0.994	0.260	0.998	1.000	0.851	0.655	0.885
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.414	0.505	0.908	0.552	0.992	0.225	1.000	0.368	0.027
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	1.000	0.678	0.983	0.977	0.992	0.975	0.864	0.907	0.867
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.331	0.989	0.698	0.290	1.000	0.289	0.883	0.102	0.086

Table 6 (continued). BGA Data. p-values of pairwise comparisons of 3-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	After 60% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.995	0.984	0.955	0.445	0.973	0.940	0.875	0.973	0.941
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.195	0.588	0.304	0.990	0.976	0.678	0.903	0.833	0.034
LR vs 140 mM BHB with 4.3 mM melatonin	0.984	0.893	0.866	0.513	0.912	0.835	0.993	0.538	0.639
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.335	0.828	0.589	0.611	1.000	0.438	0.999	0.623	0.132
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	1.000	0.989	0.995	0.997	0.997	0.999	0.753	0.824	0.941
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.335	0.938	0.688	0.693	0.995	0.245	0.784	0.165	0.274
	3 Hrs After 60% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	1.000	0.375	0.940	0.147	1.000	0.684	1.000	0.999	0.977
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.864	0.755	0.391	0.001	0.248	0.008	0.0000003	0.874	0.088
LR vs 140 mM BHB with 4.3 mM melatonin	0.827	0.438	0.918	0.001	0.047	0.0004	0.000001	0.791	0.339
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.911	0.884	0.802	0.00004	0.342	0.218	0.000002	0.949	0.272
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.873	0.987	0.696	0.0001	0.089	0.017	0.00001	0.906	0.690
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.393	0.965	0.188	0.982	0.763	0.241	0.802	0.999	0.772
	After Blood Return								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.991	0.176	0.994	0.012	0.742	0.684	0.978	0.402	0.981
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.994	0.998	0.958	0.015	0.183	0.008	0.0001	0.362	0.174
LR vs 140 mM BHB with 4.3 mM melatonin	0.858	0.854	0.745	0.046	0.024	0.0004	0.0002	0.217	0.455
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.956	0.220	0.892	0.00004	0.790	0.218	0.0003	1.000	0.378
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.986	0.426	0.905	0.0001	0.233	0.017	0.001	0.997	0.750
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.690	0.920	0.446	0.915	0.607	0.241	0.937	0.993	0.848

Table 7. BGA Data. p-values of pairwise comparisons of 1-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	Before Hypotension								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.836	0.415	0.528	0.703	0.962	0.680	0.894	1.000	0.764
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.415	0.880	0.660	0.436	0.854	0.592	0.963	0.958	0.503
LR vs 140 mM BHB with 4.3 mM melatonin	0.889	0.238	0.991	0.971	0.991	0.989	0.980	1.000	0.962
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.860	0.840	0.997	0.959	0.985	0.998	0.996	0.971	0.980
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	1.000	0.965	0.715	0.926	0.857	0.860	0.684	0.998	0.941
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.843	0.606	0.828	0.709	0.690	0.784	0.817	0.924	0.743
	After 40% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.810	0.927	0.435	0.205	0.617	0.361	0.854	0.952	0.992
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.080	0.868	0.873	0.227	0.702	0.268	0.688	0.529	0.909
LR vs 140 mM BHB with 4.3 mM melatonin	0.393	0.483	0.952	0.889	0.943	0.814	0.959	0.996	0.313
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.357	0.998	0.877	1.000	0.999	0.995	0.987	0.845	0.979
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	1.000	0.802	0.814	0.624	0.928	0.895	0.580	0.990	0.183
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.845	0.887	0.998	0.645	0.960	0.796	0.410	0.681	0.097
	10 min After 40% Blood Loss								
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.890	1.000	0.884	0.893	0.547	1.000	1.000	0.982	0.554
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.589	0.883	0.836	0.990	0.564	0.925	0.912	0.346	0.953
LR vs 140 mM BHB with 4.3 mM melatonin	0.957	0.424	0.994	0.418	0.609	0.998	0.942	0.611	0.792
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.182	0.882	0.999	0.717	1.000	0.897	0.889	0.559	0.240
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.998	0.362	0.972	0.788	1.000	0.999	0.940	0.811	0.985
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.298	0.132	0.944	0.245	1.000	0.853	0.609	0.987	0.466

Table 7 (continued). BGA Data. p-values of pairwise comparisons of 1-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
After 60% Blood Loss									
LR vs LR plus 4.3 mM melatonin	0.588	0.995	1.000	0.653	0.757	0.943	0.747	1.000	0.914
LR vs 140 mM BHB with 1.5×10^{-6} mM melatonin	0.972	0.988	0.716	0.466	0.962	0.786	0.414	0.451	0.670
LR vs 140 mM BHB with 4.3 mM melatonin	0.738	0.102	0.978	0.788	0.874	1.000	1.000	0.998	0.970
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.353	1.000	0.716	0.986	0.960	0.978	0.933	0.477	0.955
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.998	0.044	0.972	0.180	0.998	0.919	0.797	0.999	0.999
140 mM BHB with 1.5×10^{-6} mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.500	0.050	0.540	0.106	0.992	0.750	0.475	0.584	0.925
1 Hr After 60% Blood Loss									
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	0.999	0.576	0.049	0.468	0.533	1.000	0.265	0.944	0.986
LR vs 140 mM BHB with 1.5×10^{-6} mM melatonin	0.787	0.316	0.318	0.708	0.959	0.283	0.000	0.490	0.246
LR vs 140 mM BHB with 4.3 mM melatonin	0.341	0.988	0.496	0.561	0.997	0.0366	0.007	0.983	0.558
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.728	0.017	0.849	0.08758	0.860	0.262	0.015	0.795	0.141
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.294	0.803	0.803	0.0667	0.527	0.033	0.238	1.000	0.385
140 mM BHB with 1.5×10^{-6} mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.851	0.203	0.998	0.989	0.925	0.673	0.760	0.810	0.966
After Blood Return									
	pH	tHb	sO_2	K^+	Na^+	Ca^{++}	Cl^-	Glu	Lac
LR vs LR plus 4.3 mM melatonin	1.000	0.813	0.857	0.369	0.999	0.559	0.984	0.951	0.973
LR vs 140 mM BHB with 1.5×10^{-6} mM melatonin	0.982	0.990	0.200	0.151	0.800	0.008	0.062	0.924	0.226
LR vs 140 mM BHB with 4.3 mM melatonin	0.584	0.626	0.477	0.007	0.317	0.0001	0.995	1.000	0.042
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.969	0.928	0.644	0.00323	0.732	0.163	0.129	1.000	0.104
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.540	0.984	0.894	0.0001	0.264	0.003	1.000	0.968	0.017
140 mM BHB with 1.5×10^{-6} mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.809	0.777	0.983	0.466	0.805	0.270	0.143	0.950	0.813

Table 8. Regression Analysis. Cox proportional hazards table for BGA data of 3-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	pH			tHb			sO_2		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	0.396	0.529	97059	0.000	0.983	1.018	0.576	0.448	1.059
After 40% Blood Loss	0.114	0.736	0.028	0.278	0.598	2.268	0.201	0.654	0.801
10 min After 40% Blood Loss	1.027	0.311	198349	0.149	0.699	1.318	0.000	0.986	1.015
After 60% Blood Loss	0.854	0.356	0.000	0.772	0.380	1.473	0.785	0.376	1.363
3 Hours After 60% Bloos Loss	0.668	0.414	2072102	3.460	0.063	0.410	0.642	0.423	0.670
After Blood Return	0.860	0.354	0.000	0.039	0.843	0.911	0.600	0.439	1.106
	K^+			Na^+			Ca^{++}		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	0.365	0.546	0.244	2.873	0.090	0.556	0.098	0.755	35.043
After 40% Blood Loss	0.136	0.713	1.686	0.282	0.595	1.128	0.031	0.861	4.791
10 min After 40% Blood Loss	0.141	0.707	1.359	0.117	0.732	1.078	0.997	0.318	0.000
After 60% Blood Loss	0.026	0.872	0.894	2.184	0.139	1.235	0.037	0.848	2.584
3 Hours After 60% Bloos Loss	0.036	0.851	0.800	0.541	0.462	1.095	1.795	0.180	0.000
After Blood Return	0.029	0.864	1.212	1.404	0.236	0.833	1.380	0.240	4940
	Cl^-			Glu			Lac		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	0.092	0.762	1.061	1.410	0.235	1.025	0.308	0.579	1.113
After 40% Blood Loss	0.184	0.668	1.030	0.511	0.475	0.989	0.072	0.789	1.024
10 min After 40% Blood Loss	1.847	0.174	0.781	0.270	0.603	0.994	1.525	0.217	0.884
After 60% Blood Loss	1.861	0.173	1.159	0.261	0.610	0.996	0.358	0.549	1.036
3 Hours After 60% Bloos Loss	0.308	0.579	1.047	0.103	0.748	0.997	0.004	0.952	1.005
After Blood Return	0.266	0.606	0.960	0.060	0.806	1.003	0.022	0.883	1.010

Table 9. Regression Analysis. Cox proportional hazards table for 1-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	pH			tHb			sO_2		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	0.125	0.723	48.133	0.125	0.723	48.133	0.186	0.666	0.940
After 40% Blood Loss	1.669	0.196	0.000	1.669	0.196	0.000	0.633	0.426	0.680
10 min After 40% Blood Loss	0.770	0.380	0.003	0.770	0.380	0.003	0.140	0.709	1.196
After 60% Blood Loss	3.483	0.062	33458	3.483	0.062	33458	1.032	0.310	1.525
1 Hours After 60% Bloos Loss	1.343	0.247	740391	1.343	0.247	740391	0.973	0.324	1.586
After Blood Return	0.951	0.330	0.000	0.951	0.330	0.000	0.184	0.668	1.097
	K^+			Na^+			Ca^{++}		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	0.002	0.968	1.089	0.287	0.593	1.078	0.127	0.722	12.774
After 40% Blood Loss	0.494	0.482	3.403	0.033	0.856	0.966	0.279	0.598	84.496
10 min After 40% Blood Loss	0.700	0.403	0.507	0.543	0.461	1.097	0.502	0.479	0.054
After 60% Blood Loss	0.648	0.421	1.985	0.230	0.632	0.931	0.007	0.935	0.632
1 Hours After 60% Bloos Loss	2.282	0.131	2.403	1.240	0.265	0.892	0.022	0.881	2.065
After Blood Return	3.760	0.053	0.282	0.445	0.505	1.067	0.210	0.647	0.094
	Cl^-			Glu			Lac		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	2.142	0.143	1.064	5.157	0.023	0.962	0.769	0.380	1.119
After 40% Blood Loss	0.992	0.319	0.905	4.442	0.035	1.021	0.187	0.666	1.025
10 min After 40% Blood Loss	0.543	0.461	1.053	0.680	0.409	0.993	1.274	0.259	0.944
After 60% Blood Loss	0.024	0.878	1.016	0.407	0.524	1.005	2.711	0.100	1.063
1 Hours After 60% Bloos Loss	4.457	0.035	0.913	2.631	0.105	1.014	0.274	0.601	1.022
After Blood Return	2.955	0.086	1.083	3.342	0.068	0.987	0.197	0.657	0.977

Table 10. PowerLab Data. p-values of pairwise comparisons of 3-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	Before Hypotension			After 40% Blood Loss			10 min After 40% Blood Loss			After 60% Blood Loss		
	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.
LR vs LR plus 4.3 mM melatonin	0.058	0.063	0.590	0.436	0.656	0.393	1.000	0.714	0.456	0.391	0.989	0.170
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.735	0.876	0.990	0.964	0.684	0.995	0.778	0.993	0.989	1.000	0.864	0.917
LR vs 140 mM BHB with 4.3 mM melatonin	0.054	0.827	0.866	0.212	0.267	1.000	0.533	0.573	1.000	0.464	0.983	0.958
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.297	0.213	0.429	0.695	0.999	0.519	0.812	0.848	0.625	0.360	0.954	0.413
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.999	0.249	0.945	0.979	0.921	0.563	0.585	0.998	0.482	0.996	1.000	0.210
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.308	1.000	0.712	0.418	0.860	0.988	0.974	0.732	0.994	0.427	0.968	0.999
	1 Hr After 60% Blood Loss			2 Hr After 60% Blood Loss			3 Hr After 60% Blood Loss			After Blood Return		
	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.
LR vs LR plus 4.3 mM melatonin	0.957	0.943	0.709	0.363	0.559	0.625	0.389	0.396	0.536	0.046	0.414	0.411
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.031	0.242	0.148	0.059	0.072	0.266	0.275	0.061	0.332	0.306	0.105	0.090
LR vs 140 mM BHB with 4.3 mM melatonin	0.260	0.321	0.830	0.088	0.161	0.745	0.143	0.317	0.848	0.816	0.501	0.855
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.162	0.663	0.803	0.877	0.747	0.971	1.000	0.854	0.999	0.533	0.940	0.919
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.655	0.759	0.986	0.945	0.929	0.985	0.984	1.000	0.907	0.174	0.984	0.801
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.620	0.997	0.498	0.996	0.965	0.804	0.975	0.747	0.780	0.785	0.718	0.322

Table 11. PowerLab Data. p-values of pairwise comparisons of 1-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	Before Hypotension			After 40% Blood Loss			10 min After 40% Blood Loss		
	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.
LR vs LR plus 4.3 mM melatonin	0.727	0.945	0.992	0.656	0.849	1.000	0.953	0.761	0.999
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.502	0.832	1.000	0.679	0.773	0.942	0.787	0.723	0.979
LR vs 140 mM BHB with 4.3 mM melatonin	0.993	0.982	0.999	0.991	0.064	0.999	1.000	0.149	1.000
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.983	0.991	0.993	1.000	0.999	0.931	0.472	1.000	0.949
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.868	0.998	0.971	0.823	0.301	0.999	0.930	0.633	0.997
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.669	0.965	0.998	0.841	0.377	0.887	0.829	0.674	0.985
	After 60% Blood Loss			**1 Hr After 60% Blood Loss**			**After Blood Return**		
	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.	MAP	HR	Rectal Temp.
LR vs LR plus 4.3 mM melatonin	0.656	0.694	0.963	0.0001	0.333	0.702	0.0002	0.643	0.745
LR vs 140 mM BHB with $1.5x10^{-6}$ mM melatonin	0.677	0.978	0.992	0.616	1.000	0.625	0.412	0.917	0.685
LR vs 140 mM BHB with 4.3 mM melatonin	0.907	0.766	1.000	0.347	0.996	0.463	0.482	0.874	0.244
LR plus 4.3 mM melatonin vs 140 mM BHB with 1.5x10-6 mM melatonin	0.121	0.461	0.869	0.005	0.366	0.999	0.013	0.279	1.000
LR plus 4.3 mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.276	1.000	0.981	0.015	0.458	0.979	0.009	0.232	0.807
140 mM BHB with $1.5x10^{-6}$ mM melatonin vs 140 mM BHB with 4.3 mM melatonin	0.969	0.544	0.980	0.968	0.998	0.993	0.999	0.999	0.857

Table 12. Regression Analysis. Cox proportional hazards table for PowerLab data of 3-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	MAP			HR			Rectal Temperature		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	3.528	0.060	1.165	1.376	0.241	1.028	0.001	0.970	0.975
After 40% Blood Loss	0.691	0.406	0.741	0.509	0.476	1.017	0.571	0.450	0.401
10 min After 40% Blood Loss	0.784	0.376	1.088	0.196	0.658	0.986	0.999	0.318	4.950
After 60% Blood Loss	0.008	0.929	0.992	0.000	0.985	1.000	0.350	0.554	0.427
1 Hour After 60% Bloos Loss	0.481	0.488	0.969	0.729	0.393	0.922	0.190	0.663	1.436
2 Hours After 60% Bloos Loss	0.205	0.651	0.974	0.750	0.387	0.945	0.056	0.814	0.678
3 Hours After 60% Bloos Loss	0.044	0.835	1.010	0.414	0.520	1.029	0.3949	0.5297	0.295
After Blood Return	0.059	0.809	1.008	0.738	0.390	1.063	0.6335	0.4261	2.933

Table 13. Regression Analysis. Cox proportional hazards table for PowerLab data of 1-hour shock experiments at different time points through the hemorrhagic shock protocol. Statistically significant values are marked in red.

	MAP			HR			Rectal Temperature		
	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio	Chi-Square	Pr>Chi-Sq	Hazard Ratio
Before hypotension	2.258	0.133	0.958	3.069	0.080	0.986	0.155	0.694	1.197
After 40% Blood Loss	0.129	0.720	1.061	0.025	0.874	0.999	0.874	0.350	0.416
10 min After 40% Blood Loss	3.923	0.048	1.109	5.046	0.025	1.037	0.007	0.935	1.085
After 60% Blood Loss	0.212	0.645	0.960	3.713	0.054	0.987	2.207	0.137	1.899
1 Hours After 60% Bloos Loss	0.174	0.676	1.011	0.406	0.524	0.982	0.124	0.725	0.686
After Blood Return	0.619	0.432	0.982	0.281	0.596	1.017	0.003	0.958	0.947